Effects of polysulfated glycosaminoglycan and hyaluronan on prostaglandin E_2 production by cultured equine synoviocytes

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**Objective**—To investigate effects of the anti-arthritic agents hyaluronan and polysulfated glycosaminoglycan (PSGAG) on inflammatory metabolism in cultured equine synoviocytes.

**Sample Population**—Synoviocytes cultured from samples obtained from the metacarpophalangeal joints of 4 horses.

**Procedure**—Equine synoviocytes were grown in monolayer culture. Synoviocytes were stimulated with lipopolysaccharide (LPS) and simultaneously treated with various concentrations of hyaluronan or PSGAG for 48 hours. Three hyaluronan preparations were compared. Prostaglandin E_2 (PGE_2) concentrations in culture medium were measured, using radioimmunoassay.

**Results**—The highest concentrations of hyaluronan and PSGAG tested inhibited PGE_2 production.

**Conclusions and Clinical Relevance**—Clinically achievable concentrations of hyaluronan and PSGAG inhibited PGE_2 synthesis by cultured equine synoviocytes. This anti-inflammatory action may be a mechanism through which these agents exert anti-arthritic effects. The effect was obtained at concentrations that can be achieved by use of intra-articular, but not systemic, administration of hyaluronan or PSGAG. (Am J Vet Res 2000;61:499–505)

Joint disease in horses commonly is characterized by inflammation of the synovium, producing the classic signs of swelling (joint effusion), heat, and pain, causing loss of normal function and manifesting as lameness. Although synovitis often is characterized by leukocytic infiltration of the synovial membrane, synoviocytes can produce pro-inflammatory cytokines, metalloproteinases, and eicosanoids. Of the latter, prostaglandin E_2 (PGE_2) has been suggested as an important mediator of inflammation and hyperalgesia through its enhancement of vascular permeability, vasodilatory properties, and sensitization of joint nociceptors. Pharmacologic treatment of joint disease typically is directed at alleviating the signs of joint inflammation; nonsteroidal anti-inflammatory drugs (NSAID) and synthetic glucocorticoids are the most commonly used compounds. These drugs act primarily through inhibition of PGE_2 production, although glucocorticoids possess other actions, such as inhibition of induction of inducible nitric oxide synthase and the inducible isof orm of cyclooxygenase (ie, cyclooxygenase 2 [COX-2]). Pharmacologic intervention also may be directed at limiting damage to, and stimulating repair of, articular cartilage. To this end, glycosaminoglycan preparations such as polysulfated aminoglycan (PSGAG) and hyaluronan are administered intra-articularly or systemically (IV administration in the case of hyaluronan and IM administration in the case of PSGAG). Although these compounds can directly stimulate neosynthesis of cartilage matrix, it is possible that their chondroprotective effects may result indirectly from a diverse range of anti-inflammatory properties. Included in these putative anti-inflammatory actions is inhibition of PGE_2 production.

Cultured equine synoviocytes synthesize large amounts of PGE_2 in vitro when treated with lipopolysaccharide (LPS). Using the in vitro system of that study, we investigated the effects of various concentrations of hyaluronan and PSGAG on PGE_2 production by cultured equine synoviocytes. In light of claims by pharmaceutical companies of benefit of 1 hyaluronan preparation, compared with other preparations, it was of interest to evaluate a therapeutic Streptococcus zooepidemicus-derived hyaluronan preparation and a generic laboratory-grade S. zooepidemicus-derived preparation. Furthermore, claims have been made of greater therapeutic effectiveness of bacterial-derived hyaluronan, compared with rooster comb-derived hyaluronan; thus, we believed it pertinent to evaluate a preparation of hyaluronan from this latter source.

The purpose of the study reported here was to investigate possible anti-inflammatory properties of hyaluronan and PSGAG, which are used in the treatment of equine joint disease. Furthermore, the study was intended to reveal information about their mechanism of action, which remains poorly defined.

**Materials and Methods**

**Tissue specimens**—Synovial membrane was obtained from the cranial and palmar joint recesses of metacarpophalangeal (fetlock) joints of euthanatized horses. Each experiment (1 therapeutic agent/experiment) used pooled tissue specimens obtained from both metacarpophalangeal joints of the same horse. Inter-individual variation was avoided as much as possible; tissue was obtained from horses < 10 years old (as determined on the basis of examination of dentition). Synovium was only used when examination of the joints, ligaments, and tendons in the distal aspect of each limb did not reveal macroscopic signs of acute or chronic musculoskeletal...
disease. Criteria for selection were as follows: synovial membrane did not have signs of acute or chronic inflammation; articular cartilage did not have evidence of erosion or wear lines; periarticular osteophytes were not observed; synovial fluid appeared normal in quantity, viscosity, and color (ie, blood was not evident); and intra- or periarticular fractures were not evident.

Cell culture—Synovial membrane was dissected from the underlying adipose subintima, transferred to a Dulbecco modified Eagle medium containing 10% (vol:vol) heat-inactivated fetal calf serum and 1% (vol:vol) 5,000 U of penicillin/ml-5,000 µg of streptomycin/ml, and immediately subjected to enzymatic digestion (type VIII collagenase from Clostridium histolyticum, 300 U/ml, for 3 hours at 37°C). Newly released synoviocytes were seeded in 25-cm² flasks. After incubation for 48 hours, medium was changed, removing any cells not adhering to the culture flask. Flasks were maintained by changing medium at 48-hour intervals until cell replication was observed; at that time, synoviocytes were seeded in 24-well plates and grown to confluence.

Procedures involving hyaluronan—The dose of hyaluronan typically recommended for intra-articular administration in horses is 20 mg/joint. Typically, 5 to 6 ml of synovial fluid can be aspirated from a fetlock joint of a clinically normal horse. Therefore, immediately subsequent to intra-articular injection, it is possible to achieve a concentration of 3 to 4 mg of hyaluronan/ml. An inflamed joint typically will be swollen as a result of synovial effusion; administration of 20 mg of hyaluronan will result in concentrations in the synovial space that are <4 mg/ml. The rate of decrease in concentration after intra-articular administration is not known; therefore, hyaluronan was used at concentrations of 20, 200, 500, 1,000, and 2,000 µg/ml. Because addition of the highest concentration of hyaluronan (ie, 2,000 µg/ml) resulted in dilution of culture medium to 80% of normal concentration, control treatments were incorporated into the experiment.

Volumes of phosphate-buffered saline (PBS) solution equivalent to volumes for the highest concentrations of hyaluronan (ie, 1,000 and 2,000 µg/ml) were added with LPS (10 µg/ml) to culture wells. Indomethacin, a NSAID that inhibits PGE₂ synthesis, was used as a positive-control treatment.

Synoviocytes were incubated for 48 hours, using the following treatment conditions: unstimulated (medium only), 10 µg of LPS/ml and PBS solution equivalent in volume to the volumes for the highest concentrations of hyaluronan (ie, 1,000 and 2,000 µg/ml) were added with LPS (10 µg/ml) to culture wells. Indomethacin, a NSAID that inhibits PGE₂ synthesis, was used as a positive-control treatment. Synoviocytes were incubated for 48 hours, using the following treatment conditions: unstimulated (medium only), 10 µg of LPS/ml and various concentrations of PGE₂ (2, 20, 200, 2,000, 10,000 and 20,000 µg/ml), PEGAG only (20,000 µg/ml), and 10 µg of LPS/ml and a volume of PBS solution equivalent to that of 20,000 µg of PEGAG/ml.

Prostaglandin E₂ assay—Radioimmunoassay for determination of PGE₂ concentration was conducted in accordance with the general methods described by Salmon, using commercially available antisera raised against PGE₂-bovine serum albumin and tritiated PGE₂ tracer. Absolute PGE₂ concentrations of specimens were calculated by comparison with PGE₂ standards of known concentration. Specimens were diluted to ensure that values could be extrapolated from the standard curve and were subsequently corrected for the original dilution. Upper and lower limits of detection of the assay were 10 ng/ml and 50 µg/ml, respectively.

Statistical analysis—Statistical analysis was conducted, using a proprietary statistical software program. For each therapeutic agent, results represented the data of 1 experiment, with 4 replicates in each treatment group (ie, n = 4). Values were expressed as mean ± SEM. Significant differences in PGE₂ synthesis in response to various treatments were determined by use of one-way ANOVA followed by use of the Dunnett test. Significance was assigned at P < 0.05.

Results—Commercially available hyaluronan preparation—Synoviocytes incubated with 10 µg of LPS/ml produced concentrations of PGE₂ significantly (P < 0.001) higher than the basal concentrations of unstimulated cells. The LPS-indomethacin positive-control treatment caused a significantly (P < 0.001) reduced concentration of PGE₂ synthesis, compared to the following treatment conditions: unstimulated (medium only), 10 µg of LPS/ml, 10 µg of LPS/ml and 10⁻⁶ M indomethacin, 10 µg of LPS/ml and various concentrations of PSGAG (2, 20, 200, 2,000, 10,000 and 20,000 µg/ml), PEGAG only (20,000 µg/ml), and 10 µg of LPS/ml and a volume of PBS solution equivalent to that of 20,000 µg of PEGAG/ml.
with synoviocytes incubated with 10 µg of LPS/ml. Unstimulated synoviocytes incubated with the commercially available preparation at concentrations of 1,000 and 2,000 µg/ml caused decreases in PGE2 synthesis of 21 and 61%, respectively, but these values were not significantly different (Fig 1). The LPS-stimulated cultures incubated with PBS solution equivalent in volume to 1,000 and 2,000 µg of hyaluronan/ml had significantly (P < 0.001) increased PGE2 synthesis, compared with values for unstimulated cells. The LPS-stimulated cells treated with hyaluronan (20, 200, 500 and 1,000 µg/ml) did not have significant changes in the enhanced PGE2 synthesis, compared with values for LPS-stimulated cells not treated with hyaluronan (Table 1). The highest concentration of the commercially available hyaluronan preparation (2,000 µg/ml) caused a significant (P < 0.001) decrease in PGE2 production, compared with PGE2 concentrations for cells incubated with LPS alone.

**Streptococcus zooepidemicus hyaluronan preparation**—Incubation of synoviocytes with LPS significantly (P < 0.001) increased PGE2 concentrations, compared with values for basal concentrations of unstimulated cells. The LPS-indomethacin positive-control treatment significantly (P < 0.001) reduced PGE2 synthesis, compared with values for LPS alone. Synoviocytes incubated with hyaluronan (1,000 and 2,000 µg/ml) had slight, but not significant, decreases in PGE2 synthesis of 8 and 11%, respectively. Incubation of LPS-stimulated cultures with PBS solution equivalent in volume to 1,000 and 2,000 µg of hyaluronan/ml significantly (P < 0.001) increased PGE2 synthesis, compared with values for unstimulated cells; value for the cells incubated with LPS-PBS solution was similar to that of cells treated with LPS alone (Fig 2). Similar to the commercially available hyaluronan preparation, the S zooepidemicus hyaluronan preparation at low concentrations did not cause significant effects on LPS-enhanced PGE2 synthesis (Table 1). Similar to the commercially available hyaluronan preparation, however, the highest concentration of S zooepidemicus hyaluronan preparation (2,000 µg/ml) produced a significant (P = 0.007) reduction (56%) in PGE2 synthesis, compared with that for cells incubated with LPS alone.

**Rooster-comb hyaluronan preparation**—Unstimulated synoviocytes had a higher basal PGE2 synthesis than unstimulated synoviocytes in the other 2 experiments involving the use of hyaluronan. Incubation with LPS produced a comparatively weaker, nonsignificant increase in PGE2 concentration, although mean absolute PGE2 concentration (724 ng/ml) was similar to that produced by use of LPS in the other 2 experiments involving hyaluronan (Fig 3). Incubation of LPS-stimulated cultures with PBS solution equivalent in volume to hyaluronan (1,000 and 2,000 µg/ml) enhanced PGE2 synthesis similar to that of cells treated with LPS alone. In contrast to the generic S zooepidemicus and commercially available hyaluronan preparation, rooster comb hyaluronan (1,000 µg/ml) enhanced synthesis of PGE2 (increase of 83%) in unstimulated cells, which was not

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**Table 1**—Effect of various concentrations of 3 hyaluronan preparations on the percentage change in PGE2 concentration by lipopolysaccharide (LPS)-stimulated synoviocytes obtained from the metacarpophalangeal joints of clinically normal horses

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Commercially available</th>
<th>Streptococcus zooepidemicus</th>
<th>Rooster comb</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>LPS + Hyaluronan (20)</td>
<td>−12/13 8/9</td>
<td>76^a</td>
<td>76^a</td>
</tr>
<tr>
<td>LPS + Hyaluronan (200)</td>
<td>19/24 13/19</td>
<td>49/49</td>
<td>49/49</td>
</tr>
<tr>
<td>LPS + Hyaluronan (500)</td>
<td>7/19 3/19</td>
<td>19/19</td>
<td>19/19</td>
</tr>
<tr>
<td>LPS + Hyaluronan (1,000)</td>
<td>5/19 3/19</td>
<td>19/19</td>
<td>19/19</td>
</tr>
<tr>
<td>LPS + Hyaluronan (2,000)</td>
<td>−6/19 −57/19</td>
<td>24/24</td>
<td>24/24</td>
</tr>
</tbody>
</table>

Positive values reflect increased PGE2 synthesis, negative values reflect decreased PGE2 synthesis, compared with values for synoviocytes incubated with LPS alone.

*Concentration of LPS = 10 µg/ml, and hyaluronan concentrations are expressed in µg/ml.

NA = Not applicable.

^Values are significantly (a, P < 0.01; b, P < 0.001) different from those for synoviocytes incubated in LPS alone (Dunnett test, 2-tailed).

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**Figure 2**—Effect of a *Streptococcus zooepidemicus* HA preparation on PGE2 synthesis by LPS-stimulated synoviocytes obtained from the metacarpophalangeal joints of clinically normal horses. See Figure 1 for key.

**Figure 3**—Effect of a rooster comb HA preparation on PGE2 synthesis by LPS-stimulated synoviocytes obtained from the metacarpophalangeal joints of clinically normal horses. See Figure 1 for key.
Incubation of synoviocytes with LPS caused PGE2 synthesis to increase, and 2,000 µg of PSGAG/ml inhibited PGE2 synthesis by cultured human synoviocytes obtained from osteoarthritic humans had a decrease in interleukin-1α-induced PGE2 production when treated with hyaluronan at concentrations of 0.5 and 1 µg/ml. This decrease in PGE2 synthesis was also dependent on hyaluronan molecular weight. Lapine chondrocytes stimulated with interleukin-1 similarly had a decrease in PGE2 synthesis when treated with hyaluronan at similar concentrations to those used on human cells; in that study, hyaluronan had a concentration- (0.5, 1, and 2 µg/ml) and molecular weight-dependent (0.5, 1, and 2 kDa) inhibition of PGE2 synthesis. The exact molecular weight of each hyaluronan preparation used in the study reported here is not known. However, the commercially available hyaluronan preparation used in our study is of lower viscosity than other commercially available hyaluronan preparations licensed for intra-articular administration.

Analysis of the data in the study reported here failed to confirm the findings of other investigators. None of the 3 hyaluronan preparations, at concentrations up to 1,000 µg/ml, exerted inhibitory actions on LPS-induced PGE2 synthesis. However, at the highest concentration (2,000 µg/ml), 2 of the 3 preparations (commercially available preparation and S. zooepidemicus preparation) significantly inhibited but, unlike indomethacin, did not abolish PGE2 production. The reason for the failure of the rooster comb hyaluronan preparation to inhibit PGE2 synthesis at each concentration is not known. It may have been attributable to a low molecular weight of that preparation. Indeed, low concentrations of this preparation failed to inhibit and actually increased PGE2 synthesis beyond that caused by LPS alone.

A possible explanation for the increased PGE2 concentrations measured in cultures treated with rooster comb hyaluronan is that there may be pro-inflammatory contaminants in the preparation, as has been suggested elsewhere. On the basis of this supposition, it could be suggested that the enhanced PGE2 synthesis obtained in our study may have been countered only by higher concentrations of hyaluronan (1,000 µg/ml) offsetting the PGE2-stimulatory effect of potential pro-inflammatory contaminants. However, it would appear that enhanced PGE2 synthesis was not an artifact resulting from contaminated PBS solvent, because the same stock of PBS solution was used throughout the study. Furthermore, LPS-stimulated cells incubated with a volume of PBS solution equivalent to that for hyaluronan concentrations of 1,000 and 2,000 µg/ml had similar PGE2 synthesis to those cells stimulated with LPS alone.

The reduction in PGE2 production in response to 2 hyaluronan preparations (commercially available and S. zooepidemicus preparations) at the highest concentrations used (2,000 µg/ml) as a result of mere dilution of the culture medium to 80% of its original concentration can be discounted. In control cells treated with LPS in medium diluted with a volume of PBS solution equivalent to that for hyaluronan at 1,000 and 2,000 µg/ml, PGE2 concentrations were similar to those obtained in LPS-stimulated cultures maintained in full medium.

Using unstimulated hamster kidney cells, Dietmar
reported that PSGAG at concentrations of 0.03, 0.1, 0.3, and 1% reduced PGE2 concentrations in culture supernatant in a concentration-dependent manner at 16 hours (44 to 52%), and 48 hours (35 to 67%) after initiation of incubation. The PSGAG used in that study is a preparation licensed for use in humans; that preparation, similar to the preparation licensed for use in horses, contains 250 mg of PSGAG/ml. Conversion of units of concentration used in our study (µg/ml) to those used by Dietmar (percentage) indicates that reductions in LPS-induced PGE2 synthesis in cultures of equine synoviocytes were obtained at PSGAG concentrations of 0.02, 0.2, 1, and 2%, similar to those used with hamster kidney cells. The lower concentrations used in the study reported here (i.e., 0.002% and 0.0002%) did not inhibit LPS-induced PGE2 synthesis.

We were unable to account for the increase above baseline values of PGE2 synthesis by LPS-stimulated cells treated with 20 µg of PSGAG/ml. Results of experiments had little intra-experiment variation, and conditions were standardized with regard to number of cells and population. Therefore, it is unlikely that these potentially variable factors accounted for the increase in PGE2 concentration. Thus, the implication is that the increase in this treatment group may have been the result of a possible synergistic effect of LPS and PSGAG on PGE2 synthesis at that concentration of the compound. However, this is unlikely in view of the remainder of the data.

Similar to our observations of decreased PGE2 synthesis in response to hyaluronan, decreased PGE2 concentrations were obtained in cultures treated with concentrations of PSGAG similar to those estimated to be obtainable by intra-articular injection, but not at concentrations that can be achieved after IM administration.

Synoviocytes for each experiment were cultured from tissue of specific horses, which varied among experiments. All other experimental conditions were standardized, and batches of reagents and equipment were common to all experiments. Therefore, it is likely that the differences in absolute baseline and LPS-stimulated PGE2 concentrations measured in each experiment resulted from variation among horses, despite efforts to standardize selection of synovium from nonarthritic joints in horses of similar age.

In the study reported here, we measured reductions in the concentration of PGE2 in culture medium obtained from monolayer cultures of equine synovial cells. However, these experiments did not address the exact mechanism of action of hyaluronan and PSGAG at the cellular and molecular levels. Exposure of many cell types to LPS results in induction of COX-2, with consequent synthesis of PGE2 by that inducible isoenzyme of cyclooxygenase. Mechanisms of action of NSAIDs and corticosteroids in inhibiting inflammatory eicosanoid synthesis are established (inhibition of the actions of COX-2 and phospholipase A2 respectively, and, in the case of corticosteroids, inhibition of induction of the COX-2 isoenzyme as well). Given the dissimilarity in molecular structure of hyaluronan and PSGAG to corticosteroids and NSAIDs, it may be considered unlikely that hyaluronan and PSGAG exert their PGE2 concentration-lowering effects through inhibition of these enzymes. The possibility that hyaluronan inhibits the release of PGE2 into culture medium, rather than inhibiting the synthesis of PGE2, is worthy of consideration. Some investigators have suggested that the diverse actions of hyaluronan on cellular metabolism may be a consequence of its high viscosity in solution; it has been suggested that hyaluronan could physically retard the release of PGE2 from cells, as opposed to inhibiting synthesis.17,20 Similarly, it has been suggested that hyaluronan may prevent mobilization of eicosanoid precursors. Release of 14C-labeled arachidonic acid from human synovial fibroblasts in response to bradykinin and calcium ionophore is inhibited by hyaluronan in both a molecular weight- and concentration-dependent manner.24 However, authors of that study concluded that inhibition of arachidonic acid release was not associated with viscosity, because methylcellulose, a liquid similarly viscous to hyaluronan, did not inhibit arachidonic acid release.

Binding interactions of polysaccharide preparations, such as pentosan polysulfate and hyaluronan, with cell membrane molecules have received attention. Thrombospondin has been suggested as a receptor through which pentosan polysulfate may act as a matrix metalloproteinase inhibitor.19 Cellular receptors for hyaluronan have been identified, and it has been suggested that adhesion and migration of cells may be modulated through these hyaluronan receptors.26-29 Also, the capacity of hyaluronan to form complexes with phospholipids has been described recently,30 with the suggestion that administration of hyaluronan into the joint space may provide a source of binding sites for phospholipids and their metabolites (found in appreciable amounts in synovial fluid and synovial tissues of arthritic joints), which may stimulate chondrocyte catabolism. Consequently, hyaluronan may exert a chondroprotective effect by binding these pro-inflammatory (or pro-chondrodestructive) molecules. The observation in the study reported here of reduced PGE2 synthesis after incubation with hyaluronan may be explained by the binding capacity of hyaluronan, on the basis that the production of eicosanoids such as PGE2 results from metabolism of cell membrane phospholipids. Hyaluronan may bind these phospholipids in stable complexes, rendering them less susceptible to breakdown. This hypothesis has not been investigated.

Using a model of osteoarthritis in which a carpal chip was created surgically, Kawcak et al. measured PGE2 and protein concentrations in synovial fluid of horses treated by IV administration of hyaluronan. Compared with concentrations for untreated control horses, synovial fluid concentrations of PGE2 and protein were decreased in hyaluronan-treated joints. Furthermore, histologic examination of the synovial membranes of surgically explored joints revealed decreased synovial vascularity and cellular infiltration in horses treated by use of IV administration of hyaluronan, compared with surgically explored joints of untreated control horses.2 The authors of that study concluded that IV administration of hyaluronan had a positive effect in ameliorating inflammatory synovitis, although the mechanism of action of hyaluronan remains unknown. The data from our in vitro study...
warrant discussion because of results of the aforementioned in vivo study. Our calculations of the plasma and synovial fluid concentrations of hyaluronan that may theoretically be obtained following IV injection suggest that the decreased synovial fluid concentrations of PGE$_2$ observed by Kawcak et al.$^1$ are a consequence of hyaluronan's effects on leukocytes that infiltrated the synovium. On the basis of reported inhibitory effects of hyaluronan on leukocyte migration$^{13,15}$ and the identification of hyaluronan receptors on leukocytes,$^{34,35}$ it may be that the anti-inflammatory effects of hyaluronan in that in vivo experimental model of equine joint disease reside in the drug's actions on leukocytes as well as on the synovial membrane. In both instances, it would be necessary to assume that hyaluronan can exert its actions without penetrating the membrane, because this is unlikely, given its high molecular weight.


$^4$Product Information Monograph, Bayer AG, Leverkusen, Germany.


$^6$Dubelcoo's Modified Eagle's Medium, Life Technologies Ltd, Paisley, UK.

$^7$Fetal Cell Serum, Harlan Sera-Lab Ltd, Loughborouagh, UK.

$^8$Penicillin/Streptomycin Solution, Life Technologies Ltd, Paisley, UK.

$^9$Collagenase, Sigma Aldrich Co, Poole, UK.

$^{10}$Tissue Culture Flask 25-cm$^3$, Life Technologies Ltd, Paisley, UK.

$^{11}$Tissue Culture Plate 24-well, Life Technologies Ltd, Paisley, UK.

$^{12}$Product Information Monograph, Bayer AG, Leverkusen, Germany.

$^{13}$Phosphate buffered saline, Life Technologies Ltd, Paisley, UK.

$^{14}$Lipopolysaccharide, Sigma Aldrich Co, Poole, UK.

$^{15}$Indomethacin, Sigma Aldrich Co, Poole, UK.

$^{16}$Hyonate, Bayer Animal Health, Bury St Edmunds, UK.

$^{17}$Hyaluronic Acid (from S zoopidiescic), Sigma Aldrich Co, Poole, UK.

$^{18}$Hyaluronic Acid (from rooster comb), Sigma Aldrich Co, Poole, UK.

$^{19}$Product Information Monograph, Luitpold Pharmaceuticals Inc, Shirley, NY.

$^{20}$Product Information Monograph, Luitpold Pharmaceuticals Inc, Shirley, NY.

$^{21}$Adequan, Janssen Animal Health, High Wycombe, UK.

$^{22}$Anti-Prostaglandin E$_2$ Whole Antibser, Sigma Aldrich Co, Poole, UK.

$^{23}$[3H]Prostaglandin E$_2$, Amersham Pharmacia Biotech, Little Chalfont, UK.

$^{24}$Prostaglandin E$_2$, Sigma Aldrich Co, Poole, UK.

$^{25}$Systat 5, SPSS Inc, Chicago, Ill.


References


